Sero-prevalence of HTLV 1/2 and HCV in paired mother and children from Malawi as well as systematic review and meta-analysis of HTLV-1/2 data available on healthy women and children living in Africa

James Fox [1], Rob Newton [2], Marija Bedaj[2], Martin Bland [2], Nora Mutalima [2] & Fabiola Martin [2]; 1 University of Oxford, 2 University of York, UK

Background:

Very little is known about Human T lymphotropic virus type (HTLV) prevalence and co-infection with other viruses in Malawi, a very socioeconomically deprived country (GDP: $800/capita, ranking: 218/ 226 countries). The only prior study showed a 2.5% prevalence of HTLV-1 in healthy blood donors living in Malawi [1], when tested with ELISA only. We tested the HTLV prevalence and co-infection rates in the most vulnerable population group, women of reproductive age and their children, living in Malawi. At our centre stored plasma samples of 418 Malawian children and their healthy mothers were available for testing for HTLV and HCV as part of a completed cross-sectional study which looked for a link between HIV, EBV and malaria and childhood malignancies[2].

Aims:

The prevalence of HTLV-1/2 and HCV in healthy mothers of reproductive age and their children.
The transmission rate of HTLV 1/2 and HCV from mother to child.
The co-infection rate of HTLV1/2 with HCV.

Methods A:

Ethics: Approval granted by the Oxford Tropical Research Ethics Committee and the Malawian College of Medicine Research and Ethics Committee.
Study Population: 418 paired mother – child blood samples. All children (<15y) had been diagnosed with a HTLV non-related malignancy.
Place: Queen Elizabeth Central Hospital in Blantyre, Malawi.
Sampling & Testing period: 2006-2010 & 2012
Sample testing: Screening: MP Diagnostics HTLV-1/2 ELISA v4, antibodies: gp46-I [HTLV-1], gp46-II [HTLV-2], trans-membrane protein GD21 [HTLV-1/2]; MP Diagnostics HCV ELISA 3.0, anti-bodies against HCV antigens NS3 (c200), NS4, NS5 and Core (c22), MP Biomedicals, Cambridge, Cambridgeshire, UK. Confirmation: MP Diagnostics HTLV blot v2.4.HTLV-1 positive = GD21 + rgp46-I + p19 +/- p24. HTLV-2 positive = GD21 + rgp46-II + p24 +/- p19. Indeterminate = GD21, rgp46-I, rgp46-II, p19 and p24 , but did not meet HTLV-1 or HTLV-2 criteria. Negative= all other blot patterns. HCV blot 3.0 (MP Biomedicals), testing recombinant proteins (capsid, NS3, NS4 and NS5 regions) of the HCV genome.

Table 1: HTLV and HCV prevalence in mothers and children living in Malawi.

	Mothers n=418				Children		
HTLV	**Positive**	**Negative**	**Borderline**	**% Positive/418**	**Positive /28**	**% Positive/11**	**Mother**
ELISA	27	390	1	6.5	0		
HTLV-1 (3 Immunoblot/1 PCR)	**4**			**0.96**	0	0	
HTLV-2	**7**			**1.7**	**1**	9.1	HTLV-1
HTLV-1 + 2	0			0	**1**	9.1	HTLV-1
Intermediate	16			3.8	2	7.1	
False Positive ELISA	1			2.4	0	0	
Total Positive	**11**			**2.6**	**2**	**18.2**	
HCV	**Positive**	**Negative**	**Borderline**	**% Positive/418**	**Positive /3**	**% Positive/2**	**Mother**
ELISA positive	3	415		0.72	0		
Immunoblot positive	**2**			**0.48**	**1**	**50%**	**HCV**

Figure 1: Forest plot showing prevalence of HTLV among women as reported in four regions of Africa.

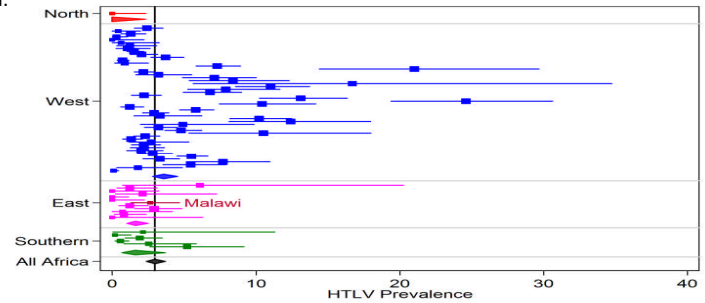

Table 2: Results of meta-analysis by different African regions.

Region	Countries (number of samples)	Prevalence %	95% CI
North	Egypt (1)	0.0	0.0 to 2.3
West and Central	Benin (3), Central African Republic (1), Cameroon (6), Democratic Republic of Congo (7), Gabon (9), Gambia (2), Guinea Bissau (10), Ghana (3), Guinea (1), Ivory (1), Nigeria (5), Senegal (2)	3.6	2.8 to 4.5
East	Djibouti (2), Eritrea (2), Ethiopia (2), Malawi (1), Mozambique (4), Somalia (1)	1.6	1.0 to 2.5
Southern	Namibia (1), South Africa (5)	1.6	0.7 to 3.7
All Africa		3.0	2.4 to 3.7

HTLV DNA testing: Blood samples of indeterminate participants were DNA tested using generic HTLV primers and according to previously published methodology.

Methods B:

Following an exhaustive PubMed search 66 publication were included, which reported HTLV prevalence in healthy women (antenatal care, blood donors, healthy volunteers and sex workers) and had performed a confirmatory test on ELISA positive samples. The confidence intervals for individual studies were by the exact binomial method and the combined estimate by random effects meta-analysis using the logistic transformation. Where there were no observed HTLV cases (0 prevalence) 0.5 was added to frequencies for the meta-analysis.

Results A:

2.6% of healthy women were HTLV carriers and remarkably 1.7% were HTLV-2 carriers. Two children were HTLV carriers: one child was dually infected with HTLV 1 + 2 and was the child of a HTLV-1 positive mother. The second child was infected with HTLV-2 , although the mother was HTLV-1 carrier.
HCV antibody prevalence was very low among healthy women (0.48%) but the child of one of the two infected women was a HCV antibody positive.
HTLV/HCV co-infection was not observed.

Results B:

Our analysis revealed an overall 3% prevalence of HTLV in healthy African women. This varied considerably depending on geographical area and reporting frequency per country and grouping of women. HTLV was more prevalent in older women and sex workers (data not shown). HTLV-2 was reported very rarely (10 reports), and at a much lower rate (estimated 0.89%, range 0.1-3.8) than our observation in Malawi. Prevalence data from North Africa was scarce. Three groups reported on mother to child transmission which was very high in Gabon (18%).

Discussion:

Our data detected a high prevalence of HTLV-2 in healthy women living in Malawi. This was unexpected and has not been reported before. Horizontal transmission of HTLV is possibly the cause of sero-positivity of the children. The low prevalence of HCV in Malawi is reassuring although sampling bias cannot be excluded.
Our systematic review highlights large gaps in HTLV surveillance reporting and confirms previous reports of high prevalence of HTLV in women.

References:
1 **Candotti D**. et al. Serological and molecular screening for viruses in blood donors from Ntcheu, Malawi: high prevalence of HIV-1 subtype C and of markers of hepatitis B and C viruses. J Med Virol. 2001 Sep;65(1):1-5.
2 **Mutalima N.** et al. Associations between Burkitt lymphoma among children in Malawi and infection with HIV, EBV and malaria: results from a case-control study. PLoS One. 2008 Jun 18;3(6):e2505.

Contact: Fabiola.martin@hyms.ac.uk

#2241